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Gas chromatography for detection of citrus infestation by fruit fly larvae (Diptera: Tephritidae)☆

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ABSTRACT

Tephritid fruit flies are serious economic pests worldwide. As larvae, they feed and develop within the pulp of host fruits, making infestation difficult to detect by visual inspection. At U.S. ports of entry, incoming produce shipments are checked for infestation by manually cutting open a small sample of fruit and searching for tephritid larvae. Consequently, there is a need for more sensitive, high-throughput screening methods. This study evaluated gas chromatography (GC) as a potential technology for improved detection of hidden infestation. Grapefruits (*Citrus* × *paradisi* Macfad.) infested with immature stages of the Caribbean fruit fly *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) were examined to determine if infested fruit emitted a chemical profile distinct from that of non-infested fruit. Peaks identified by GC analysis were grouped into three classes. Chemicals detected in similar quantities in all samples, or slightly elevated in infested samples, were regarded as non-diagnostic background volatiles. Chemicals highly elevated after oviposition, during the last instar exit stage, and in experimentally-pierced fruit were interpreted to be indicators of citrus peel injury, and included D-limonene and β-ocimene. Chemicals elevated exclusively in the larval infestation stages were considered indicators of feeding damage and potentially diagnostic of infestation, and included hexyl butanoate and an unidentified compound. The peaks associated with injury and feeding were also detectable with a portable ultra-fast GC analyzer that required less than 80 s per sample. Further studies will investigate the potential application of these results for development of a rapid, non-destructive screening method for detection of tephritid infestation.

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1. Introduction

Tropical tephritid fruit flies are invasive pests that impact fruit production and export worldwide. Current U.S. appropriations for exotic fruit fly risk management programs are over $57 million per year (USDA-APHIS, 2006). Primary threats to U.S. agriculture include the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), which has a global distribution and numerous hosts (Liquido et al., 1991), and the *Anastrepha* species, which occur throughout the American tropics and subtropics (Aluja, 1994). The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is established in Florida and is regarded as a quarantine pest of grapefruit, *Citrus* × *paradisi* Macfad. (Nguyen et al., 1992; Greany and Riherd, 1993). Other *Anastrepha* species pose an invasive threat due to proximity of populations in Mexico and the Caribbean basin (White and Elson-Harris, 1992). In addition, the large volume of foreign produce entering U.S. ports creates potential pathways for tephritid entry and spread (Kendra et al., 2007 and references therein). It has been estimated that an infestation of *C. capitata* in the U.S could cost as much as $1.5 billion yearly due to export sanctions, lost markets, treatment costs and crop losses (USDA-ARS, 2005).

Due to the high economic impact of tephritid pests, much attention has been focused on development of trapping systems for detection and monitoring of adult populations (Heath et al., 1995; Casaña-Giner et al., 2001; IAEA, 2003; Thomas et al., 2008). However, improved methods are critically needed for detection of the immature stages as well. Adult females have well-developed ovipositors that insert eggs beneath the skin of host fruits. Larvae feed and develop concealed within the pulp, making infestation difficult to detect. At U.S. ports of entry, quarantine inspectors currently check incoming produce shipments by examining a small sample (typically 2% or less) of fruit for external signs of pest boring/feeding, and if suspicious, by slicing open the fruit to search for tephritid larvae (USDA-APHIS, 2010). Efficacy of visual

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inspections is questionable, especially for first instar larvae which are clear to pale white and only 2–3 mm in length. Gould (1995) estimated that only about 35% of grapefruits infested with *A. suspensa* were detected by trained agricultural inspectors. If not subjected to appropriate quarantine treatments, infested fruit may be distributed to consumers and/or discarded directly into the environment. Due to the risk of pest introduction should infested fruit evade detection, there is great demand for more sensitive, high-throughput screening methods for detection of tephritid larval infestation.

This study evaluates gas chromatography (GC) as a potential technology for improved detection of hidden insect infestation. It is well documented in the literature that insect herbivory can elicit changes in host plant chemistry and volatile emissions (reviewed in Karban and Baldwin, 1997; Howe and Jander, 2008). It also has been shown that chemical changes can occur within host fruit as a result of insect infestation (Boevé et al., 1996; Hern and Dorn, 2001; Carrasco et al., 2005). In this study we examined citrus infested with *A. suspensa* to determine if infested fruit emitted a detectable chemical profile distinct from that of non-infested citrus. Samples of headspace volatiles were collected at various stages of infestation and chemical analysis was performed with several types of GC equipment. Since the primary goal was development of a rapid screening protocol for "signature chemicals", the majority of analyses were performed with a rapid (9 min) GC separation method. To evaluate the efficacy of separation with this rapid method, and to identify the volatile chemical components, a slower (25 min), high resolution GC separation was performed in combination with mass spectral analysis. In addition, we conducted a preliminary evaluation of a portable ultra-high speed GC analyzer for detection of these same chemicals using a method requiring less than 80 s for sampling and chemical analysis.

2. Materials and methods

2.1. Infestation and sample preparation

A. suspensa were obtained from a laboratory colony maintained at the USDA-ARS, Subtropical Horticulture Research Station in Miami, FL. All flies were of known age and reared under the following conditions: 25 ± 2 °C, 75 ± 5% RH, and a 12:12-h (L:D) photoperiod (Kendra et al., 2006). Adults of mixed-sex (~1:1 sex ratio) were housed in screen rearing cages (30 cm × 30 cm × 30 cm) and provisioned with water (released from agar blocks) and food (refined cane sugar and yeast hydrolysate, 4:1 mixture) *ad libitum* prior to collection for fruit infestation. Approximately 3500 mature (10–12 d old, presumed mated) females were collected by aspiration from the rearing cages and placed in each of two infestation cages (94 cm × 51 cm × 51 cm) constructed from PVC frames covered with mesh pollination bags (Delstar Technologies, Middletown, DE). Each cage contained 50 ripe Florida-grown grapefruit (*Citrus* × *paradisi* cv. Marsh Red, obtained from a local natural foods market) arranged in a single layer. Oviposition was allowed for 24 h, and then the fruit was removed and rinsed with distilled water to remove the fly excreta (and any potential volatiles it may have liberated).

Half of the infested fruit was randomly divided into five groups for chemical sampling at different stages of infestation: egg, first instar, second instar, mid-third instar, and exiting third instar (final instar larvae exit the host fruit and enter a wandering stage prior to pupation in the soil). Two control treatments were also sampled; they consisted of non-infested fruit and mechanically injured fruit that were pierced with a tack five times to simulate oviposition wounds (tack length approximated length of *A. suspensa* ovipositor, 2.0 mm). The remaining half of the infested fruit was used to monitor progress of larval development. At 2–3 d intervals, several grapefruits were cut open to determine the larval instar and to estimate the level of infestation. Each segment was opened and the pulp separated and gently pressed to dislodge larvae. The albedo, pulp and juice were then examined under a microscope to detect larvae. Until the time of chemical sampling, all fruit treatments were held in the laboratory at the same environmental conditions used for insect rearing. Following chemical collections, each sampled fruit was cut open and examined (as above) to document the developmental stage and the number of insects present.

2.2. Volatile collections and chemical analysis

Grapefruits were placed individually into 3.85 L jars with Teflon-lined lids fitted with short thru-hull ports (Swagelok; Solon, OH) and allowed to equilibrate for 30 min at 22 °C. Headspace volatiles were collected using Solid Phase Microextraction (SPME) with a 100 μm polydimethylsiloxane-coated (non-bonded) fiber (Supelco; Bellefonte, PA). A sample was collected by inserting the SPME fiber through the port and exposing the fiber to headspace volatiles for 2 min adsorption.

Volatile profiles were obtained using a rapid separation method on a Trace™ GC (ThermoFisher; Waltham, MA) equipped with a DB-5 column (20 m × 0.18 mm × 0.18 μm) and a flame ionization detector (FID, 300 °C). Chemicals were injected by thermal desorption (splitless injector, 250 °C for 2 min) from the SPME fiber directly into the GC. Helium was used as the carrier gas at a constant flow rate of 0.05 mL s^{-1}. The temperature program consisted of an initial oven temperature of 50 °C which was increased after injection at a rate of 0.583 °C s^{-1} up to 220 °C, and then held at 220 °C for 4 min. Total run time was 9 min. Chemical analysis with the rapid GC method was performed on five replicate fruits per treatment (five infested and two control treatments).

To identify component peaks, additional SPME collections (as above) were analyzed by GC–mass spectrometry (GC–MS). Adsorbed chemicals were injected into a 5975B GC/MSD (Agilent; Santa Clara, CA) equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas. The temperature program consisted of an initial oven temperature of 50 °C which was increased at 0.167 °C s^{-1} to 130 °C, followed by a second ramp from 130 to 210 °C at 0.333 °C s^{-1}. Total run time was 25 min. MSD source was set at 230 °C, quadrapole at 150 °C, and scans were recorded for mass range of 50–650 amu. Three replicate fruits per treatment were analyzed by GC–MS, and component peaks were identified using the NIST/EPA/NIH mass spectral library (NIST05) and confirmed by retention time and mass spectra of known standards.

A portable chemical profiling system incorporating an ultra-high speed chromatography column (zNose® Model 4200; Electronic Sensor Technology; Newbury Park, CA) was used for comparative analysis of selected samples. Headspace volatiles were collected by inserting the unit's intake needle into the sample port and allowing for 30 s adsorption (at a flow rate of 0.05 mL s^{-1}) onto an internal Tenax® trap. Chemicals were injected by thermal desorption and separated on a DB-5 column (1 m × 0.25 mm) using helium carrier gas, a temperature ramp of 40–175 °C, and a surface acoustic wave (SAW) detector. Total run time from sample collection to GC separation was 79 s.

2.3. Statistical analysis

Prior to analysis, peak area of each chemical was normalized relative to the internal SPME standard for that GC run. For comparison of volatile profiles from different treatments, each chemical peak was evaluated separately by one-way analysis of variance (ANOVA) using Proc GLM (SAS Institute, 2001). Significant ANOVAs were fol-

Table 1
Normalized GC peak area (mean ± SD) of volatiles obtained by SPME collections and rapid (9 min) GC analysis from grapefruits (*Citrus* × *paradisi* cv. Marsh Red) infested with *Anastrepha suspensa* and non-infested controls. $N = 5$ per treatment.

RT (min)	Infested treatments					Controls		*F*	*P*
	Egg	1st instar	2nd instar	3rd instar	Exit holes	Non-infest.	Injured		
3.18[a]	6136.4 ± 4661.1 a	2321.1 ± 2518.0 a	1914.5 ± 1587.0 a	715.2 ± 403.6 a	3507.2 ± 54.51.7 a	22.4 ± 50.2 b	7021 ± 3951.3 a	19.51	<0.0001
3.48[a]	66.4 ± 38.2 bc	329.8 ± 333.9 a	182.9 ± 129.8 ab	49.3 ± 52.3 c	63.6 ± 45.8 bc	45.1 ± 25.1 c	64.1 ± 39.6 bc	3.38	0.0124
3.61[b]	30.6 ± 14.2 a	2.2 ± 5.0 c	14.3 ± 20.4 bc	8.5 ± 10.7 bc	12.2 ± 13.2 ab	1.6 ± 3.6 c	6.7 ± 6.5 bc	3.71	0.0077
3.80[b]	19.1 ± 6.2 c	175.9 ± 101.9 a	76.9 ± 64.6 b	59.9 ± 55.0 bc	62.8 ± 42.1 bc	2.6 ± 5.8 d	50.4 ± 16.6 bc	9.03	<0.0001
4.00[a]	5.6 ± 3.4 a	27.5 ± 34.2 a	21.1 ± 21.3 a	9.5 ± 3.8 a	4.7 ± 3.7 a	0.0 ± 0.0 b	1.9 ± 4.2 b	10.58	<0.0001
4.22[a]	2.6 ± 5.8	3.3 ± 3.2	4.2 ± 3.6	12.2 ± 22.5	8.4 ± 8.9	0.0 ± 0.0	21.4 ± 36.4	1.95	NS
4.33[a]	8.4 ± 9.8 a	15.7 ± 6.5 a	6.3 ± 5.5 a	4.2 ± 4.9 a	2.5 ± 3.5 a	0.0 ± 0.0 b	13.6 ± 20.4 a	3.34	0.0131
4.53[b]	16.8 ± 19.4 abc	34.3 ± 28.9 a	25.8 ± 20.7 ab	9.7 ± 10.2 bcd	5.7 ± 4.3 cd	1.1 ± 2.6 d	15.9 ± 14.4 abc	3.69	0.0079
4.62[a]	31.5 ± 9.5 a	38.1 ± 20.6 a	46.6 ± 25.1 a	30.7 ± 23.2 a	26.1 ± 8.6 a	0.0 ± 0.0 b	20.1 ± 26.4 a	11.56	<0.0001
4.80[b]	42.6 ± 16.8 ab	63.4 ± 26.1 a	24.3 ± 10.9 bc	18.0 ± 12.2 c	23.4 ± 11.3 bc	20.7 ± 18.3 bc	57.5 ± 33.7 a	3.92	0.0057
4.89[b]	17.7 ± 7.7 a	19.4 ± 3.8 a	8.4 ± 3.7 ab	6.4 ± 4.7 b	8.7 ± 5.9 ab	0.6 ± 1.3 c	10.1 ± 11.8 b	5.94	0.0004
5.06[a]	62.4 ± 26.7	255.9 ± 123.2	146.7 ± 69.1	112.0 ± 80.1	113.7 ± 60.0	28.4 ± 24.6	101.9 ± 76.4	1.19	NS
5.18[b]	24.1 ± 8.3 ab	35.3 ± 17.0 a	19.0 ± 8.4 ab	16.7 ± 12.0 bc	18.7 ± 9.1 ab	6.7 ± 5.9 c	16 ± 7.2 bc	3.49	0.0106
5.28[a]	23.9 ± 11.5 a	8.8 ± 5.8 ab	8.4 ± 5.2 ab	4.5 ± 3.6 bc	7.1 ± 7.1 ab	0.0 ± 0.0 c	12.6 ± 17.8 ab	4.82	0.0017
5.45[a]	13.6 ± 5.8 a	6.1 ± 6.7 ab	1.9 ± 4.3 bc	1.9 ± 2.6 bc	1.0 ± 2.3 bc	0.0 ± 0.0 c	8.3 ± 12.0 ab	3.32	0.0135
5.69[a]	15.2 ± 23.9	3.1 ± 4.3	3.0 ± 2.8	0.9 ± 1.3	1.1 ± 2.5	0.0 ± 0.0	1.8 ± 4.0	1.6	NS
5.81[a]	34.4 ± 27.2 a	9.2 ± 4.6 a	6.5 ± 2.4 a	4.6 ± 2.8 a	6.2 ± 3.1 a	0.0 ± 0.0 c	2.1 ± 2.9 b	12.06	<0.0001

[a] Means followed by the same letter are not significantly different (LSD mean separation test on $\log(x+1)$ transformed data, $P < 0.05$; non-transformed means presented).
[b] Means followed by the same letter are not significantly different (LSD mean separation test on square-root$(x+0.05)$ transformed data, $P < 0.05$; non-transformed means presented).

lowed by least significant difference test (LSD, $P < 0.05$) for mean separation. The Box-Cox procedure, which is a power transformation that regresses log-transformed standard deviations ($y+1$) against log-transformed means ($x+1$), was used to determine the type of transformation necessary to stabilize the variance before analysis (Box et al., 1978).

3. Results and discussion

3.1. Level of infestation

Grapefruit dissections made immediately after chemical sampling indicated there were no significant differences in level of infestation among the five infested treatments ($F = 1.55$; df = 4, 24; $P = 0.226$). Mean (±SD) number of insects per fruit detected for each treatment was as follows: 17.0 (±20.5) eggs, 25.8 (±16.1) first instar larvae, 44.2 (±24.9) second instar larvae, 43.4 (±24.0) third instar larvae, and 47.6 (±22.1) exit holes from prepupal third instar larvae. Due to the difficulty with detection of the very early stages (Gould, 1995), the numbers reported for eggs and first instar larvae are likely to be under-representative of the actual infestation level in those two treatments. Dissections performed on the control fruits (non-infested and mechanically injured treatments) confirmed that they lacked immature stages of *A. suspensa*.

3.2. Identification of volatile constituents

There were 17 major peaks separated by the rapid GC analysis of grapefruit volatiles (Table 1). For all but three peaks, there were significant differences in quantities represented in the different treatments. One peak (RT 3.18 min) was greatly elevated in both the infested treatments and the injured fruit as compared to the non-infested controls. High resolution GC–MS analysis revealed that this broad peak represented two closely-eluting chemicals, D-limonene and β-ocimene (Fig. 1, peaks 5 and 6, respectively), and both chemicals were elevated with fruit infestation/injury. The highest levels were detected from fruit mechanically injured and fruit punctured by oviposition (Fig. 2A). Levels decreased with each progressive larval instar, apparently due to wound healing of the epidermis and epicarp (flavedo) of the grapefruit (Mulas et al., 1996). Levels again spiked when late third instar larvae began to exit the fruit and reinjure the peel. D-limonene and β-ocimene are known terpene constituents of citrus peel, and they comprise up to 93% and 2.7% composition, respectively, of the peel oils in 'Marsh' grapefruits (Attaway et al., 1967). The large limonene/ocimene peak was therefore interpreted to be an indicator of citrus peel damage, specifically a puncture wound (whether inflicted mechanically or by the female ovipositor).

Two broad peaks (RT 3.48 and 3.80 min, rapid method) were markedly elevated in the infested treatments relative to the non-infested controls and the mechanically injured fruit (Table 1). High resolution GC–MS showed that the 3.48 peak consisted of n-nonanal and an unidentified chemical (Fig. 1, peaks 7 and 8, respectively), but only the unknown compound was associated with larval infestation. The 3.80 peak consisted of hexyl butanoate (Fig. 1, peak 10), ethyl octanoate (Fig. 1, peak 11), and possibly another chemical (Fig. 1, unlabeled peak preceding peak 10), but hexyl butanoate was the primary chemical elevated with infestation. Hexyl butanoate (=hexyl butyrate) is a fruit ester, a major component of the aroma from apples *Malus domesticus* Borkh. (Matich et al., 1996), pears *Pyrus communis* L. (Argenta et al., 2003) and passion fruit *Passiflora edulis* Sims (Werkhoff et al., 1998), but in this study it was detected at very low levels in healthy intact grapefruit. Under natural conditions, hexyl butanoate emissions increase with the progression of fruit ripening, apparently an ethylene-

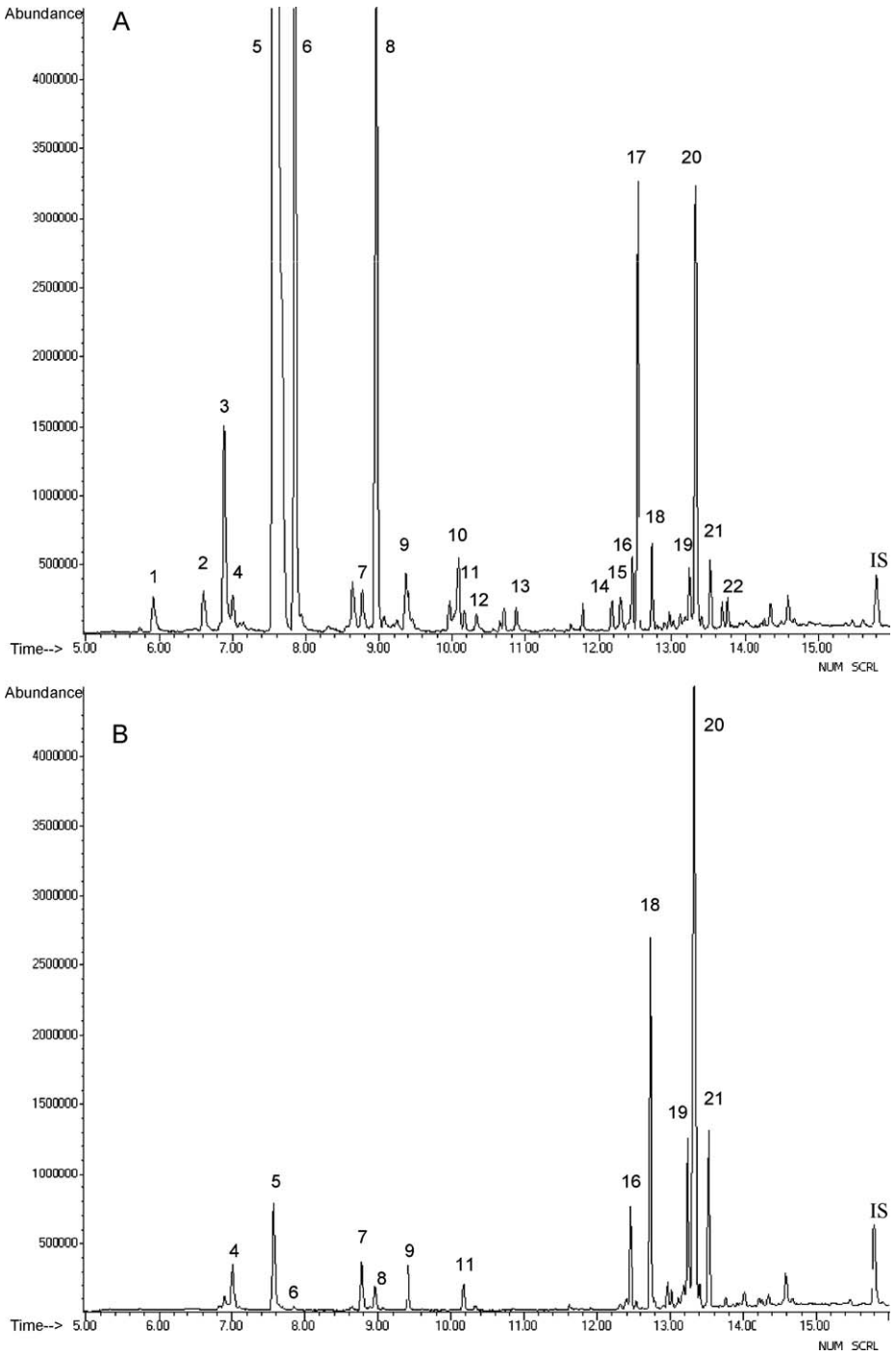

Fig. 1. High resolution (25 min) GC analysis of headspace volatiles obtained by SPME collections from grapefruit (*Citrus* × *paradisi* cv. Marsh Red). (A) Fruit infested with first instar larvae of the Caribbean fruit fly, *Anastrepha suspensa.* (B) Non-infested fruit. Peak identifications are as follows: 1. α-pinene, 2. sabinene, 3. β-myrcene, 4. ethyl hexanoate, 5. D-limonene, 6. β-ocimene, 7. n-nonanal, 8. unknown, 9. limonene oxide, 10. hexyl butanoate, 11. ethyl octanoate, 12. decanal, 13. isoamyl hexanoate, 14. eugenol, 15. hexyl hexanoate, 16. β-elemene, 17. methyl eugenol, 18. β-caryophyllene, 19. 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene, 20. valencene, 21. α-panasinsen, 22. nerolidol, IS: internal standard.

mediated response (López et al., 2007). With both hexyl butanoate and the unidentified chemical, the highest levels were observed in fruit infested with first instar larvae (Fig. 2B and C), the stage of infestation most difficult to detect by visual inspection, and levels declined with subsequent instars. The ripening process would not account for the observed decrease in hexyl butanoate levels over time. This pattern of induced volatile emissions has been documented in another fruit commodity. Apples infested with larvae of the codling moth (*Cydia pomonella* L.) initially emitted high levels of esters and α-farnescene; the highest values were recorded from fruit infested with first instar larvae, but with time the amounts decreased to levels equivalent to that from healthy fruits (Hern and Dorn, 2001). These elevated chemicals (in both commodities) may be interpreted as indicative of injury within the pulp or albedo layers as a result of larval feeding, but it is unclear why the levels would decrease during the later (larger) larval instars. Of the two peaks associated with tephritid-infested grapefruits, the unknown compound appeared to be the better candidate as a signature chem-

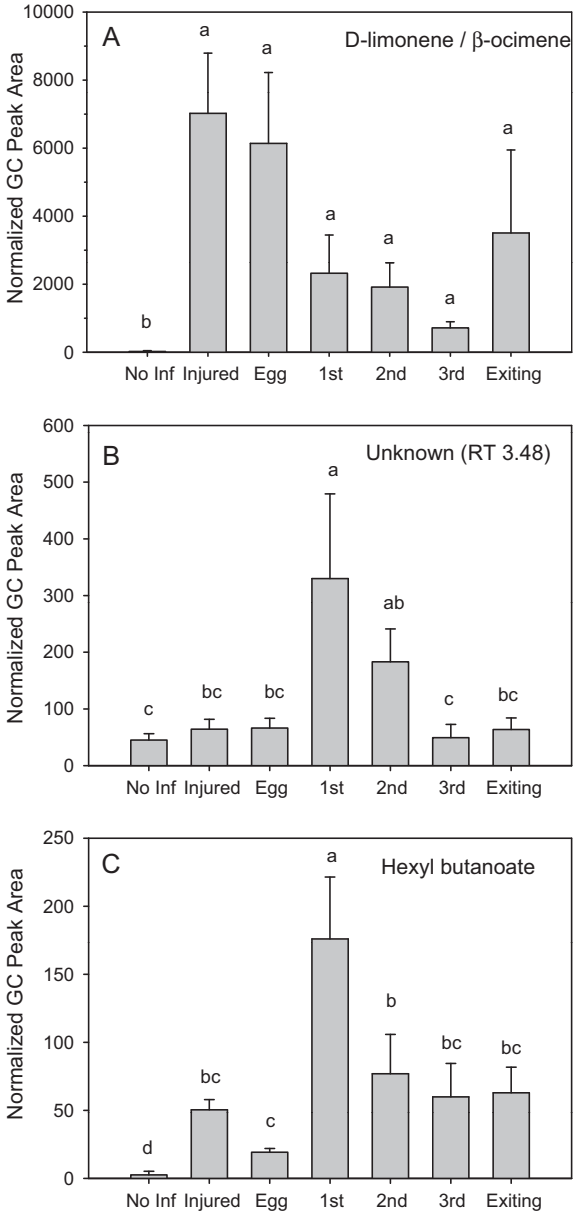

Fig. 2. Mean (±SE) quantity of (A) D-limonene/β-ocimene, (B) an unknown compound with retention time of 3.48 min, and (C) hexyl butanoate as determined by SPME collections and rapid (9 min) GC analysis of headspace volatiles from grapefruits (*Citrus* × *paradisi* cv. Marsh Red) that were non-infested, mechanically-injured, or infested with various immature stages of the Caribbean fruit fly, *Anastrepha suspensa*. Quantities are expressed as normalized GC peak areas relative to an internal SPME standard. Peaks for the unknown compound (B) and hexyl butanoate (C) contain small amounts of co-eluting n-nonanal and ethyl octanoate, respectively, but high resolution GC indicated neither was elevated with infestation. Bars topped with the same letter are not significantly different [LSD mean separation test on log $(x+1)$ transformed data (A and B) or square-root$(x+0.05)$ transformed data (C), $P<0.05$; non-transformed means presented].

ical for larval infestation. It was a much larger peak and there was better separation from neighboring peaks (Fig. 1A). Unfortunately, there was no match for this compound within the NIST mass spectral library. Additional work is needed to determine the chemical identity and source (host fruit, insect larvae, or microbial origin).

There were several other chemical peaks associated with fruit infestation and/or injury. These included isoamyl hexanoate (Table 1, RT 4.0; Fig. 1A, peak 13), co-eluting eugenol and hexyl hexanoate (Table 1, RT 4.53; Fig. 1A, peaks 14 and 15, respectively), and methyl eugenol (Table 1, RT 4.62; Fig. 1A, peak 17). Although signifi-

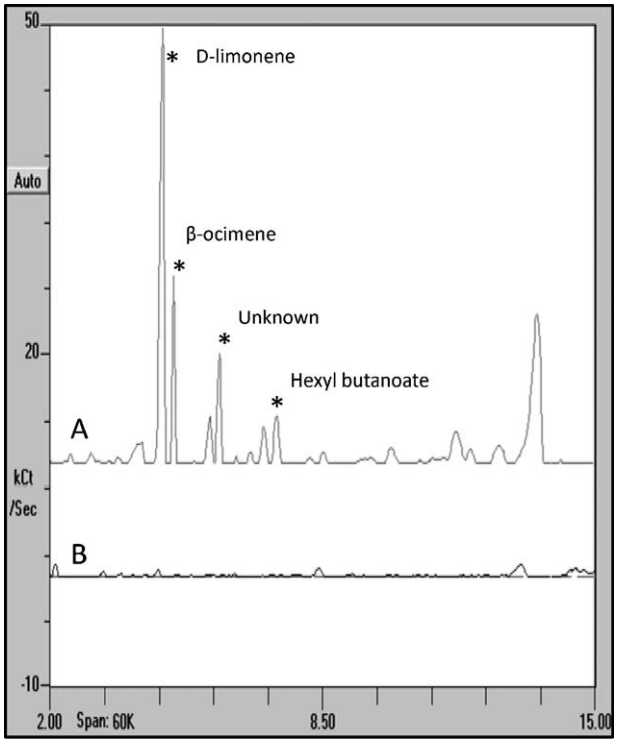

Fig. 3. Ultra-fast (79 s) GC analysis of headspace volatiles from grapefruit (*Citrus* × *paradisi* cv. Marsh Red). (A) Fruit infested with first instar larvae of the Caribbean fruit fly, *Anastrepha suspensa*. (B) Non-infested fruit. Chemical peaks indicative of fruit injury and/or infestation are labeled. The peak for hexyl butanoate contains a small amount of co-eluting ethyl octanoate, but high resolution GC indicated it was not elevated with infestation.

cantly higher in the infested/injured fruit compared to non-infested fruit, the first three chemicals were detected at fairly low levels (i.e., small peaks), and with rapid GC separation the methyl eugenol co-eluted with β-elemene, a chemical found in significant quantities in non-infested fruit (Fig. 1B, peak 16). Therefore, it was concluded that none of these additional chemicals would serve well as reliable indicators of infestation. It was also noted that high resolution GC–MS detected α-pinene, sabinene, and β-myrcene (Fig. 1A, peaks 1–3, respectively) in infested fruit but not in the non-infested controls. Although potentially diagnostic of infestation, these small, fast-eluting chemicals were not resolved well by the rapid GC separation. The first clear peak eluting with the rapid GC method was the large limonene/ocimene peak. As with limonene, elevated levels of pinene and myrcene have been shown to be correlated with wounded citrus fruit (Droby et al., 2008).

Initial evaluation of the ultra-fast GC unit (Fig. 3) indicated that it was capable of detecting some of the same signature chemicals that were identified with high resolution GC. Compared to the 9 min separation method, the ultra-fast method actually gave better resolution of the lower molecular weight compounds. There was good separation between D-limonene and β-ocimene. Likewise, there was good resolution between n-nonanal and the unidentified chemical associated with infestation. Hexyl butanoate was also detectable with the ultra-fast method, but it co-eluted with ethyl octanoate as was observed with the 9 min GC method. The ultra-fast method gave poor resolution of the higher molecular weight compounds (RT > 9.0 s), but none of the peaks of interest eluted within that region.

Altered composition of host fruit odors (volatile profiles) as a result of tephritid larval infestation has been demonstrated previously, primarily through studies addressing host-seeking behavior in the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead)

(Hymenoptera: Braconidae). Though chemical attractants were not identified, Eben et al. (2000) used y-tube olfactometers to show that female *D. longicaudata* responded preferentially to odors from grapefruit and mango infested with *Anastrepha ludens* or *Anastrepha obliqua* versus non-infested control fruits. Carrasco et al. (2005) reported similar preferences in female *D. longicaudata* from bioassays that used hexanic and methanolic extracts from infested mangos as compared to non-infested or mechanically damaged fruit. Comparative GC–MS analysis of the extracts indicated that there were both qualitative and quantitative differences in chemical content among the three mango treatments. Several compounds were elevated with infestation, including 3-carene, limonene, terpinolene, and α-gurgenene, and one compound, 2-phenylethyl acetate, appeared to be unique to mangos infested with *A. ludens*.

4. Conclusions

Results of our study and that of Carrasco et al. (2005) indicate that there are GC-detectable volatile chemicals associated with tephritid infestation of fruit commodities. With infested grapefruits, the chemicals can be distinguished as those indicative of citrus peel injury and those correlated with larval feeding (pulp/albedo injury). Of the chemicals identified, none appeared to be insect produced, but rather natural fruit volatiles occurring at higher levels than normal. Elevated levels of D-limonene and β-ocimene are only indicative of puncture wounds or other external damage to the grapefruit peel. However, if these two chemicals are accompanied by elevated levels of hexyl butanoate and the (as of yet) unidentified compound, this volatile profile is potentially diagnostic of citrus infestation. Preliminary tests indicate that these chemical signals, emitted from fruit with early stages of infestation, are detectable with the portable zNose® unit.

In recent years, sensitive chemical detection technology (zNose® and other forms of electronic nose) has been applied successfully in a variety of postharvest situations for early detection of insects and pathogens. These include detection of stink bugs (Hemiptera: Pentatomidae) and boll damage in cotton (Henderson et al., 2010), evaluation of fungal disease severity and stage of ripeness in mango (Li et al., 2009), and detection of lesser grain borers (Coleoptera: Bostrichidae) and extent of feeding damage in wheat (Zhang and Wang, 2007). If insect-infested commodities consistently release unique chemical profiles, this signature can be exploited to provide the basis for improved pest detection. Further evaluation of the tephritid/citrus system is needed to (1) determine the sensitivity of larval detection by ultra-fast GC methods, (2) assess the applicability of these methods to other species of Tephritidae, other citrus hosts, and hosts of different ages (since background volatiles vary over time due to ripening and storage), and ultimately (3) apply this technology toward development of rapid, more sensitive, non-destructive screening methods for detection of tephritid infestation at ports of entry.

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